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Origin of Meiotic Nondisjunction in Drosophila Females

by Rhoda F. Grell*

Meiotic nondisjunction can be induced by external agents, such as heat, radiation, and chemicals, and by internal genotypic alterations, namely, point mutations and chromosomal rearrangements. In many cases, nondisjunction arises from a reduction or elimination of crossing over, leading to the production of homologous univalents which fail to co-orient on the metaphase plate and to disjoin properly. In some organisms, e.g., Drosophila and perhaps man, distributive pairing (i.e., a post-exchange, size-dependent pairing) ensures the regular segregation of such homologous univalents. When a nonhomologous univalent is present, which falls within a size range permitting nonhomologous recognition and pairing, distributive nondisjunction of the homologues may follow. Examples of nondisjunction induced by inversion heterozygosity, translocation heterozygosity, chromosome fragments, radiation, heat, and recombination-defective mutants are presented.

Most detectable aneuploidy in Drosophila, as well as in humans, occurs as a consequence of nondisjunction at the first meiotic division. In analyzing the origin of nondisjunctional gametes, one aspect frequently overlooked is the close dependence of nondisjunction on reduced chiasma frequency. Reductions arise either from decreased exchange or less often from premature chiasma resolution. Homologs that become univalents from either cause are expected to assort independently of one another and lead to the production of gametes carrying both or neither homolog one-half of the time. When univalents occur, some organisms, such as Drosophila, possess a mechanism to avert nondisjunction and its unfortunate consequences by providing a second opportunity for homologs to pair and disjoin regularly. This mechanism is called distributive pairing (*1*).

In the present paper I want to discuss principally the relationship between crossing over and disjunction as we have found it to exist in Drosophila. The discussion will necessarily entail a description of the properties of the distributive mechanism since it is an integral component of the segregation behavior of chromosomes in the Drosophila oocyte; it may have relevance for an understanding of the origin of some human aneuploidy as well, since there is reason to suspect the existence of a similar mechanism in the human oocyte (*2-4*). The common denominator, then, for many cases of meiotic nondisjunction, particularly those which do not involve spindle defects or alterations, appears to be reduced exchange. Reductions may be induced by genotypic alterations, namely, chromosomal rearrangements and point mutations, or they may be induced by external agents such as radiation, chemicals, and heat.

Among chromosome rearrangements, the heterozygous inversion is recognized as the most effective method of reducing exchange between homologs. First, we can examine the effect an exchange and disjunction of a fairly small inversion strategically placed in a chromosome. The example I have chosen is Inversion (1) AB which lies medially in the X chromosome and occupies about 1/6 of its euchromatic length. When In (1) AB is present in heterozygous condition (Fig. 1*a*), crossover analyses show that it reduces exchange from its normal level of 62% between the tip and carnation to ~18%, i.e., ~a 70% reduction (R. F. Grell, unpublished data). Strand analysis by Weinstein's procedure (*5*) reveals that noncrossover X tetrads are increased from 5 to 65%. If X univalents are present 65% of the time, random assortment should lead to X nondisjunction over 30% of the time. Instead it is found to occur less than one-half of 1%; 0.45% to be exact (R. F. Grell,

*Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

unpublished data). The reason X nondisjunction remains very low is that X univalents undergo a post-exchange pairing with one another which permits them to segregate almost as regularly as if they were bound by a chiasma.

This situation prevails as long as the X chromosomes, or any pair of homologs, are the only univalents present in the distributive pool. (Here we ignore the small fourth chromosomes which will be discussed later.) Figure 1*b* illustrates what happens when a univalent heterolog is also present part of the time. The heterolog utilized in this study is a second chromosome carrying the multiple inversions Glazed. Its homolog is involved in a reciprocal translocation with chromosome 3 and as such is almost always part of a crossover complex. Again crossover data are compiled and converted into tetrad frequencies. The rearrangements in chromosome 2 have, through the interchromosomal effect, increased X exchange so that the frequency of X univalents is reduced to 26% and chromosome 2 is estimated to be a univalent 57% (R. F. Grell, unpublished data). Since the X's and the 2 univalents must be present in the same oocyte for nonhomologous pairing to occur, the opportunity for such pairing exists in 14.8% of the oocytes (26%·57%). The frequency of X nondisjunction is found to be 4.8%, which is one order of magnitude greater than that

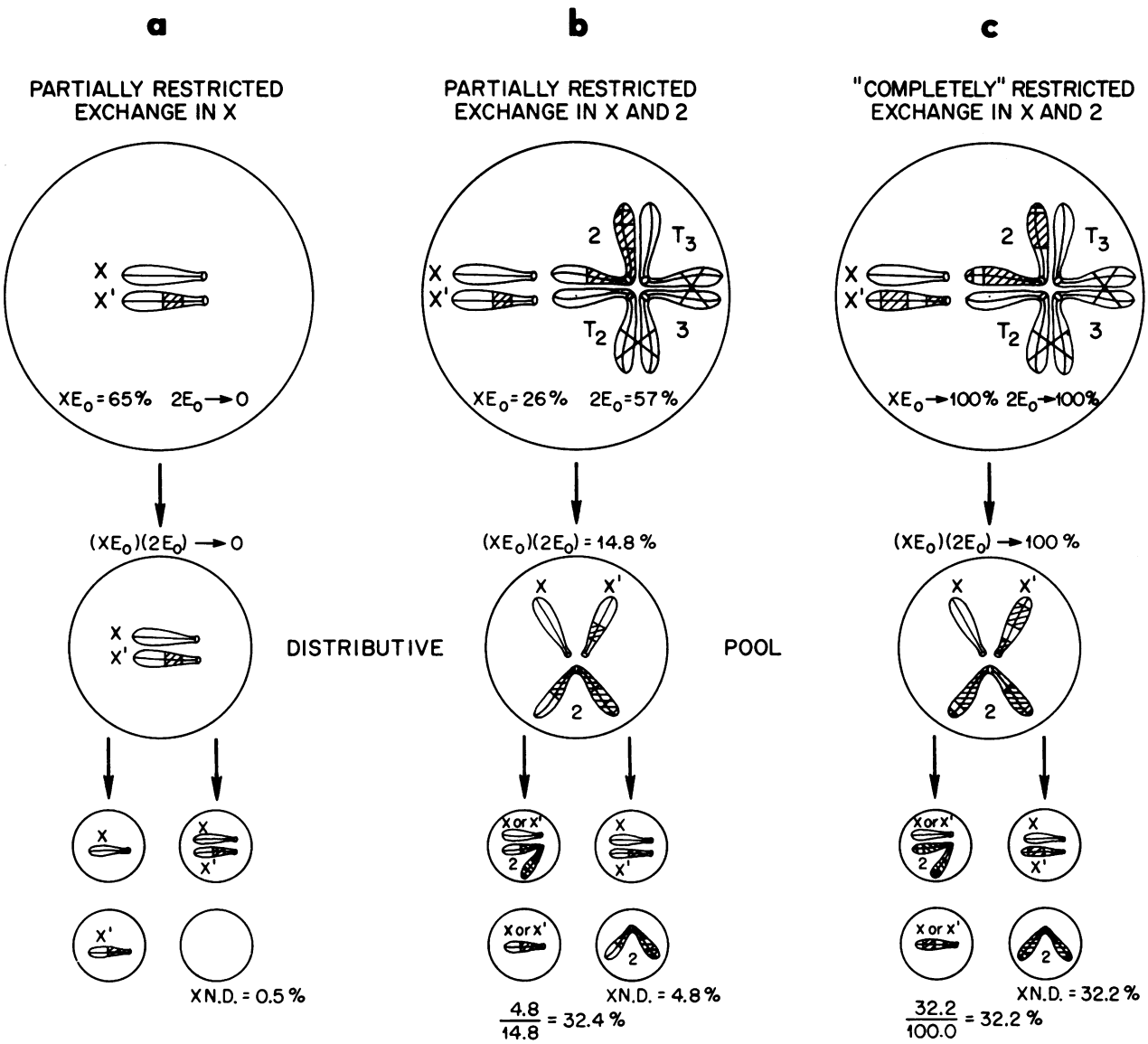

FIGURE 1. Effect of inversion heterozygosity on X nondisjunction. Female genotypes are (*a*) In (1)AB/+, (*b*) In(1)AB/+;In(2LR)Gla/T(2;3)101, (*c*) Ins(1)dl-49, B^{M1}/+;SMI/T(2;3)101. E_0 denotes noncrossover tetrad; N.D. indicates nondisjunction.

observed in the absence of the univalent 2.

Finally, crossing over can be virtually eliminated in the X's and the 2 by the use of more complicated rearrangements as shown in Figure 1*c*. If X and 2 noncrossovers are now assumed to approach 100%, opportunity for nonhomologous pairing will occur in close to 100% of the oocytes. X nondisjunction in this situation is found to increase by another order of magnitude to 32% (R. F. Grell, unpublished data). If we return to the second situation and instead of calculating X nondisjunction on the basis of the entire population we calculate it on the basis of the 14.8% when the opportunity for nonhomologous pairing exists, we find it also occurs 32% of the time (4.8/14.8). In other words, the univalent second chromosome induces the X's to nondisjoin about one-third of the time and this value is constant whether the opportunity for induction occurs in 15% of the oocytes or 100% of the oocytes. Thus aneuploidy resulting from X univalents which are normally present in ~5% of *D. melanogaster* oocytes, as well as aneuploidy arising from inversion polymorphism, characteristic of many Drosophila species, can be efficiently averted by the distributive mechanism. With the introduction of a new rearrangement into the genome, distributive pairing of nonhomologs will again lead to aneuploidy but to a lesser degree than would be found in the absence of the mechanism.

Translocations are a second type of chromosomal rearrangement which affect exchange and disjunction. Semisterility is a well known property of translocation heterozygotes, and the reduction in fertility derives from the formation of aneuploid gametes. The probability of recovering a euploid gamete from a translocation heterozygote is greatly enhanced if all members of the translocation complex are maintained as a crossover multivalent until metaphase I. The retention of the multivalent is in turn related to the location of the breakpoints and the probability of exchange between each translocated element and its normal homolog.

I have carried out a series of experiments using three different translocation heterozygotes to demonstrate the dependence of aneuploid frequency on the position of the breakpoints and the chance of chiasma formation (*6*). The genotype of the translocation-bearing females is shown in Figure 2. In each case, a reciprocal 3;4 translocation is present with one break in the right arm of chromosome 3 and a second break in the small fourth chromosome. The three translocations are distinguishable by the increasingly distal position of the break in 3R whereas the break in 4 remains constant at 101F in the salivary gland chromosomes. In each case, the translocation produces a larger element T_3, which carries

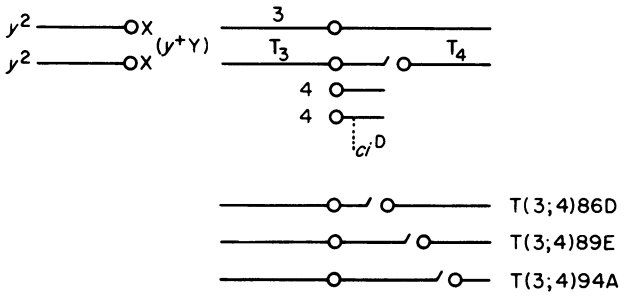

FIGURE 2. Genotype of females carrying a 3;4 translocation heterozygously. Breakpoints of the three translocations used are indicated below the genotype.

the intact left arm, the centromere, and the proximal portion of the right arm of chromosome 3, the latter capped with the euchromatin of chromosome 4; and a smaller element T_4, which carries the centromeric region of chromosome 4 to which the distal portion of 3R is attached. As the break in 3R becomes more distal, the length of T_3 increases and the length of T_4 shows a corresponding decrease. To avoid the production of inviable haplo-4 progeny, the females carried two free 4's as well as the translocated 4. A marked Y chromosome, which is always a univalent, served as a tool to assess the frequency that a univalent translocation element pairs distributively with the Y and segregates from it. The frequency of Y, T segregation in excess of the 50% expected on the basis of random assortment of the Y became a measure of the degree of aneuploidy characteristic of each translocation. This follows because segregation of the Y from one translocation element is independent of segregation of chromosome 3 from the other translocation element, and to produce a viable zygote the oocyte must possess both elements. Hence, one-half of Y, T associations lead to lethal aneuploids. [The T_4 element of T (3;4) 94A is infrequently recovered as a duplication.] The frequency that T_3 and T_4 are univalents was arrived at in the usual way by measuring exchange in each element followed by tetrad analysis of the crossover data. Sisters of identical genotype, except for the lack of a Y chromosome, served as a control to ensure that distributive pairing of the Y and a translocated chromosome did not alter exchange values.

Table 1 gives the crossover data and the frequencies of noncrossover tetrads (E_0) for the T_3 and T_4 element of each translocation as well as Y, T association frequencies based on Y, T segregation. First, it is evident that crossingover in T_3 and in T_4 is unaltered by the presence of the Y demonstrating that distributive pairing of the Y and the T_3 or T_4 element does not affect the frequency that either element undergoes exchange with the normal 3. Second, the frequency of the E_0 for T_3 and T_4 is unaltered by Y, T associations. Third, crossingover decreases and E_0

Table 1. Frequency of crossingover and E_0 tetrads for the T_3 and T_4 components of the three T(3;4) translocation heterozygotes and the Y,T association frequency derived from the Y,T segregation frequency for each translocation.

		Crossingover, %			E_0 tetrads, %			Y,T association, %[b]	Y,T segregation, %
		T_3	T_4	Total	T_3	T_4	Total[a]		
86 D									
	O	55	41	96	5	20	24	—	—
	Y	52	41	93	5	22	26	32	66
89 E									
	O	53	15	68	7	70	72	—	—
	Y	52	18	70	11	64	68	68	84
94 A									
	O	53	0.9	54	12	98	98	—	—
	Y	55	1.8	57	10	96	96	84	92

[a] $T_{33_0} + T_4E_0 - (T_3E_0 \cdot T_4E_0)$.
[b] Calculated from the formula $a = 1 - (2n)$, where a = association and n = nondisjunction.

frequency increases as the break in 3R becomes more distal. Fourth, the E_0 frequency, calculated from tetrad analysis, corresponds very closely with Y, T association, calculated from Y, T segregation frequencies, indicating that virtually all noncrossover translocation elements pair with and segregate from the Y; and conversely that virtually all crossover translocation elements assort independently with the Y. Since a univalent translocation element, whether it segregates from the Y or assorts independently, is recovered with its reciprocal element one-half of the time, the amount of aneuploidy is not altered by Y, T associations. The critical feature determining the degree of aneuploidy and lethality is the frequency of T_4E_0. This frequency ranges from 20% to 98%, depending on the location of the breakpoint and the probability of chiasma formation. The frequency that a T_3-3-T_4 trivalent is maintained until metaphase I is inversely related to T_4E_0 frequency. The correlation between T_4E_0 and lethality is reflected in the decrease in the average number of progeny per female over a 7-day period with the three translocation heterozygotes (Table 2).

Table 2. Number of progeny/female (7 day brood).

	No. translocation	T(3;4)86D/+	T(3;4)89E/+	T(3;4)94A/+
Observed				
O		248	152	144
Y		236	188	111
Expected[a]				
Y	350[b]	266	210	189

[a] Calculated from Y,T associations (Table 1) assuming an equal number lead to lethality.
[b] Assuming 50 progeny/♀/day as normal and that the total expected with no translocation (350) includes the lethal fraction.

The last structural alteration I will consider is a free duplication. The experiment to be described (7) shows how a small fragment can disrupt the regular segregation of a pair of homologs, and at the same time it demonstrates that chromosome size is a controlling factor in the disruptive process.

Up to this point the small fourth chromosomes have been ignored, although they are always noncrossovers. Their regular segregation, despite the absence of a chiasma, led to the supposition that a special device, peculiar to the fours, might exist to prevent their nondisjunction. This experiment made use of a series of X duplications (i.e., X chromosomes deleted of all but their tip and a variable amount of proximal heterochromatin) of well determined size. Their length varied from ≤ 0.3 to over 3 times the size of the fourth chromosome, the latter arbitrarily valued at one. Each duplication was introduced into a female carrying two dominantly marked fourth chromosomes and the frequency of four nondisjunction was measured among her progeny to determine if it was affected by the presence of the duplication. Figure 3 shows the results. The smallest duplication (~0.3) has no effect; but as duplication size approaches four size, nondisjunction of the fours steadily increases. With a duplication very close in size to the fours (1.1), a peak value of 37% four nondisjunction is reached. As the duplications become larger than the fours, nondisjunction decreases. This experiment demonstrated that the fours can be induced to nondisjoin with a very high frequency if a heterolog of proper size is available. Although duplications were utilized here, any fragment within the proper size range, such as one resulting from a very asymmetrical translocation, e.g., Robertsonian translocation, would be equally effective. The closer the size resemblance between the heterolog and the four, the higher the nondisjunction

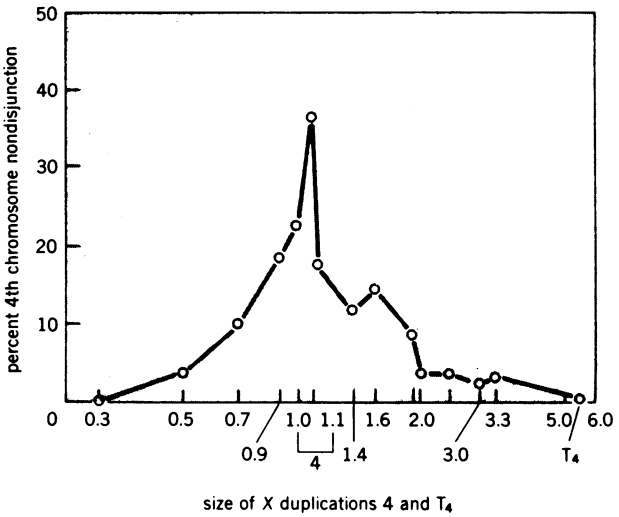

FIGURE 3. Induction of distributive nondisjunction of fourth chromosomes by X duplications. Highest nondisjunction value (37%) is reached with a duplication closest in size to chromosome 4, and lowest with duplications farthest in size from the 4. From Grell (*8*).

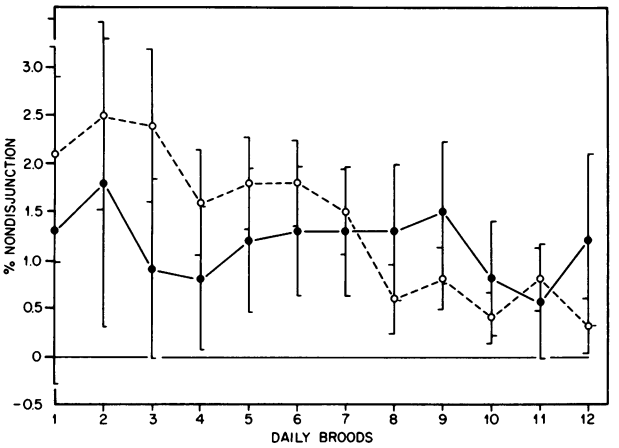

FIGURE 4. Percentage of nondisjunction (with 95% confidence limits) of the 2 X chromosomes and of the X duplication and the fourth chromosome (size ratio 10:1, respectively) for the first 12 daily broods from females irradiated shortly after ecolosion with 4000 R of x-rays: (●) X; (○) X dp, 4 (*12*).

frequency. Beyond this it became evident that distributive pairing is the mechanism which normally ensures regular segregation of the fours. Thus, like genomes with inversion polymorphism, genomes possessing small chromosomes, which infrequently or never crossover, benefit greatly from the distributive mechanism.

Recombination is, of course, under genetic control, and point mutations can play a major role in the induction of nondisjunction. For example, among an array of mutants selected for their ability to induce nondisjunction of the X and fourth chromosomes at meiosis I in the Drosophila oocyte the primary effect of the mutant in nearly all cases was found to be a reduction in crossingover, and the observed nondisjunction was a secondary consequence of this reduction (*9*). To illustrate the degree of aneuploidy possible with a point mutation, I will use a mutation that we recently recovered (*10*) which I call *rec-1*^{6}. In homozygous condition this mutant reduces crossingover in the X chromosome from ~60% to less than 3%. At the same time X nondisjunction escalates from ~0.1% to 30%. As we have seen, if only X exchange were affected, X nondisjunction would remain very low. It is very high because *rec-1*^{6} reduces autosomal exchange in a similar fashion so that X and autosomal univalents are simultaneously present in the distributive pool with a high frequency.

Turning to external agents, the ability of x-rays to induce nondisjunction (*11*) was recognized prior to Muller's discovery of x-ray induction of mutations. Over 50 years later, the mechanism of radiation-induced nondisjunction is still poorly understood, and its properties depart markedly from those associated with radiation-induced mutation. As shown in Figure 4, the brooding pattern reveals no stage sensitivity. A dose of 4000 R of x-rays, delivered to stage 7 oocytes which are recovered in the first-day brood, produces about the same frequency of X nondisjunction as does the same dose delivered to oogonial cells recovered in the 12-day brood (*12*). On the other hand, nondisjunction of two regularly segregating nonhomologs, in this case an X duplication and a fourth chromosome, does display stage sensitivity. A significant decrease is observed following the 7-day brood and corresponding to the transition to oogonial cells (Fig. 4).

Since X homologs are generally chiasma-bound whereas disjoining heterologs are not, a subsequent study examined the role of exchange on radiation-induced nondisjunction (*13*). Females homozygous or heterozygous for two X inversions (X-exchange tetrads estimated at 90% and 24%, respectively) were irradiated with 4000 R within 6 hr of eclosion. No significant difference in nondisjunction between the two genotypes is observed in the first seven daily broods, where brood 1 represents stage 7 oocytes. Incomplete tests of stage 8-14 oocytes by use of 500 R suggest that, unlike the extreme sensitivity of mature oocytes to lethal induction, nondisjunction remains roughly proportional to dose in both the homozygotes and heterozygotes.

Traut (*14*) has reported that 1000 R represents a threshold dose below which X nondisjunction is not increased above the spontaneous rate.

The studies of Parker and Williamson (*15*) have shown that one source of radiation-induced non-

disjunction comes from induced exchange between heterologs. If such an exchange behaves in the same way as a crossover between homologs, the distributive model would predict that the heterologs would be excluded from the distributive pool and that they would disjoin. The homologs of the translocated chromosomes remaining in the pool would assort independently of the interchange, and as a consequence nondisjunction of homologs would follow. Tests of these predictions have shown that the experimental results fulfill the expectations. It is unclear how large a contribution to radiation-induced nondisjunction is made in this way.

Elevated temperature is also capable of inducing nondisjunction in the Drosophila oocyte (*16*). Unlike radiation, for which the germ cells display little if any stage sensitivity, X-nondisjunction frequency is only affected if heat treatment is initiated during a limited sensitive period. Further, its effect is restricted to the X chromosomes. A heat treatment of 35°C (10°C above control) given between 114 and 132 hr of development to a well-synchronized population of females induces significant increases in X nondisjunction. At these times the oldest germ cells, which are those recovered for study, are at a late oogonial or very early oocyte stage.

Table 3 shows how nondisjunction frequency is modified by length of treatment. A 24-hr treatment initiated at 114 hr causes a rise to 2.4%, i.e., about 20 times the control (0.1%). A 12-hr treatment initiated at the same time gives only 0.7%. No increase is observed with initiation at 132 hr or later, indicating that treatment continued beyond this time is ineffective.

In conformity with most previously considered cases, production of E_0 X tetrads appears to be a prerequisite for induction (Table 3). Support for this conclusion comes from the failure to detect a single case of heat-induced autosomal nondisjunction coupled with the finding that heat fails to produce autosomal E_0 (*17*).

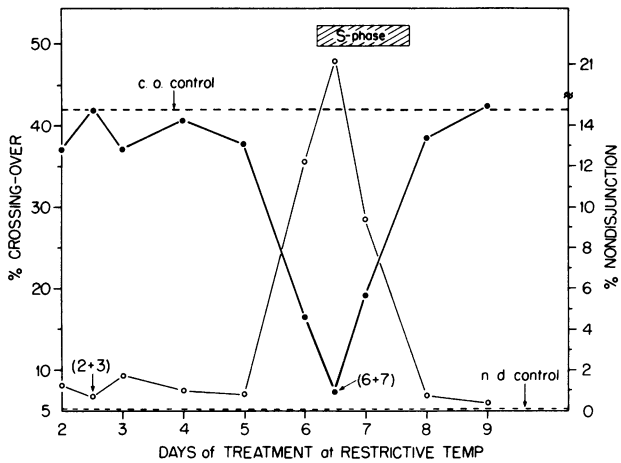

FIGURE 5. Effect of restrictive temperature (31°C) on crossingover and nondisjunction on different groups of *rec-1*[26] females heat-treated for 24 hr on sequential days of development from day 2 to day 9 or heat treated for 48 hr on days 2 and 3 or on days 6 and 7: (●) crossingover; (○) nondisjunctions; (- - -) control; S-phase denotes premeiotic S; c.o. control denotes crossingover control; n.d. control denotes nondisjunctoin control (*18*).

The final case illustrates the way that an internal change, in this case a point mutation, and an external factor, heat, can interact to increase nondisjunction and aneuploidy. This experiment (*18*) made use of a temperature-sensitive recombination mutant called *rec-1*[26] which is an allele of the mutant I discussed earlier. At 25°C, *rec-1*[26] reduces X recombination to about 80% of normal; at 31°C, it is reduced to ~10% of normal.

Reduction is extremely stage-specific. As shown in Figure 5, heat treatment of synchronized populations of females carrying this mutant, on successive days of development, produces no significant effect if given to immature germ cells on days 2, 3, 4, or 5 or to oocytes on days 8 or 9. Sensitivity is confined to days 6 and 7 when the oocytes are undergoing DNA synthesis. A treatment on day 6 or 7 reduces X

Table 3. Effect of elevated temperature (35°C) on frequencies of X chromosome crossingover, E_0 tetrads, and nondisjunction.

Treatment post egg laying, hr	Treatment, hr	Total map units	E_0 X tetrads, %	X nondisjunction, %
Control	0	68.5	7.1	0.1
114-138	24	37.9[a]	45.0	2.4
114-126	12	55.8[a]	22.6	0.7
120-132	12	51.8[a]	30.8	0.7
126-138	12	65.2	20.9	0.7
132-144	12	82.4[a]	8.9	0
138-150	12	80.3[a]	2.5	0

[a]Significant increase or decrease from control value, $p \leq 0.05$.

exchange to 16% and 19%, respectively, whereas a treatment which includes both days 6 and 7 causes a reduction to ~7%. Further dissection locates sensitivity between 126 and 162 hr, virtually coincident with the limits of premeiotic DNA replication.

Examination of the nondisjunction curve shows that it is reciprocally related to the crossover curve. When crossingover is high on days 2 to 5 and days 8 and 9, X nondisjunction is low; when crossingover is reduced on days 6 or 7, nondisjunction increases; and when treatment includes both days and crossingover is at its minimum, nondisjunction reaches its peak value.

The high incidence of X nondisjunction implies both the presence of noncrossover major autosomes in the distributive pool and the occurrence of autosomal nondisjunction leading to additional aneuploid products. The degree of aneuploidy is indicated by the great reduction in the number of progeny per female from ~16 in the control to ~4 when the treatment covers the sensitive period.

In summary, meiotic nondisjunction in Drosophila originates in a variety of ways. Of those reviewed here, all but one, namely, radiation-induced nondisjunction, depend on the abnormal presence of univalent chromosomes in the distributive pool. The univalents are generally a consequence of reductions in crossing over arising from genetic or environmental changes or from interactions between the two.

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